Supplementary Figure 1: Comparison between SingleSplice and a baseline method. (a) Receiver operating characteristic (ROC) curve for the baseline method (choosing an arbitrary cutoff value to separate significant ratio change from no change). Each point on the curve indicates the true positive and false positive rates for a particular choice of the cutoff value. The performance of SingleSplice is indicated as a single point rather than a curve because there are no tunable parameters. (b) Plot showing the distributions of ratio variance for true negative (black) and true positive (green) test cases. The dotted line indicates the best cutoff ($c = 0.05$) according to the ROC curve in the previous panel. (c) Ratio variance for test cases in which both spike-in transcripts have mean expression less than or equal to 10 RPKMs. Note that in this range of expression levels, the fixed cutoff derived from the full set of spike-ins will show poor specificity, biasing the baseline method toward calling low expression pairs as alternatively spliced. (d) Ratio variance for test cases in which both spike-in transcripts have mean expression no smaller than 1000 RPKMs. Note that in this range of expression levels, the fixed cutoff derived from the full set of spike-ins will show poor sensitivity, biasing the baseline method away from calling high expression pairs as alternatively spliced.

Supplementary Figure 2: Evaluation of the influence of read depth on alternative splicing detection. This plot shows the number of ASM paths detected in each cell as a function of the number of reads in that cell. Note that there is an approximately linear relationship between read depth and number of ASMs detected. The number of reads in the Treutlein experiment is typical for single cell RNA-seq experiments, while the Buettner dataset has unusually deep coverage. The Buettner experiment also sequenced a larger number of cells, so we selected a random subset of cells the same size as the Truetlein dataset to make the two sets of cells as comparable as possible.

Supplementary Table 1: Differences between bulk RNA-seq and single cell RNA-seq that require the development of methods tailored to single cell RNA-seq analysis

Supplementary File 1: Genes with isoform usage variation exceeding what is expected based on technical noise alone.

Supplementary File 2: Genes with isoform usage changes linked to the cell cycle

Supplementary File 3: GO terms enriched in genes with isoform usage changes linked to the cell cycle